Effects of a 48-h Fat Infusion on Insulin Secretion and Glucose Utilization

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To determine the effects of prolonged elevation of plasma free fatty acids (FFAs) on insulin secretion, we infused Liposyn II (4.3 μmol · kg^{-1} · min^{-1}) plus heparin (0.4 U · kg^{-1} · min^{-1}) intravenously into six healthy volunteers for 48 h. Another six volunteers received saline infusions and served as control subjects. In all 12 subjects (11 men and 1 woman), plasma glucose was clamped at ~8.6 mmol/l. Liposyn/heparin infusion resulted in a 9.4-fold increase in plasma FFA concentration (from 132 to 1,237 μmol/l), a 46% increase in insulin secretion rates (from 241 to 352 pmol/min, $P < 0.05$) (determined by deconvolution of plasma C-peptide concentration), and a 30% decrease, during the initial 24 h, in the rate of glucose infusion needed to maintain hyperglycemia (from 55.5 to 39.1 μmol · kg^{-1} · min^{-1}, $P < 0.02$). This decrease disappeared during the second 24 h. In summary, we found that physiologically elevated plasma FFAs *1*) potentiated glucose-stimulated insulin secretion for 48 h and *2*) initially caused peripheral insulin resistance that disappeared during the 2nd day, probably as a result of elevated circulating insulin levels. We conclude that in healthy volunteers under hyperglycemic conditions, fat infusion produced insulin resistance that was compensated for after ~24 h by persistent hypersecretion of insulin. *Diabetes* 44:1239–1242, 1995

Acute stimulation of insulin secretion by free fatty acids (FFAs) has been well established (1–6). Sako and Grill (7) reported recently, however, that prolonged (48 h) exposure to elevated FFAs produced biphasic insulin responses. After 3 and 6 h of high FFAs, glucose-stimulated insulin release from isolated perfused rat pancreas was increased, whereas it was inhibited after 24 and 48 h (7). The same authors showed that incubation of rat islets with palmitate or oleate for 48 h markedly reduced glucose-stimulated insulin synthesis and secretion (8). These findings could be of considerable clinical relevance, particularly in view of the recent demonstration from our laboratory and others that elevation of blood FFA concentration produced peripheral insulin resistance in healthy volunteers (9–11) and in patients with non-insulin-dependent diabetes mellitus (NIDDM) (12) and in view of the possible augmenting effects of FFAs on hepatic glucose production (11). Hence, increased FFA levels may be able to produce all of the major pathogenetic abnormalities commonly found in patients with NIDDM, namely, peripheral insulin resistance, increased hepatic glucose production, and decreased insulin secretion.

Inhibition of insulin secretion after prolonged elevation of plasma FFA, however, has been shown only in vitro (in isolated perfused pancreas and in isolated islets) and only in rats (7,8). To establish physiological or pathophysiological significance, similar effects of prolonged FFA elevation need to be demonstrated in vivo and in humans. It was the aim of this study, therefore, to examine in healthy volunteers the effects of prolonged (48 h) infusion of fat on prehepatic insulin secretion and on peripheral glucose utilization.

RESEARCH DESIGN AND METHODS

Subjects. Twelve healthy normal-weight volunteers (11 men and 1 woman) were studied. Their ages, weights, heights, and body mass indexes (BMIs) are shown in Table 1. Their weights were stable for at least 2 months, and their diets contained a minimum of 250 g/day carbohydrate for at least 2 days before the studies. Informed written consent was obtained from all after explanation of the nature, purpose, and potential risks of these studies. The protocol was approved by the Institutional Review Board of Temple University Hospital.

Experimental design. To continuously stimulate insulin secretion, plasma glucose was clamped at ~8.6 mmol/l in all 12 subjects for 48 h. Six subjects received, in addition, a continuous infusion of fat plus heparin, which raised their plasma FFA levels. The other six subjects received saline, which resulted in low FFA levels, and served as control subjects.

All subjects were admitted to Temple University Hospital's General Clinical Research Center on the evening before the studies. The studies began at ~8:00 A.M. after an overnight fast with the subjects reclining in bed. A short polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was placed into a contralateral forearm vein for blood sampling. This arm was wrapped with a heating blanket (~70°C) to arterialize venous blood. We have found in previous experiments that this results in near-identical arterial and venous forearm glucose concentrations.

Hyperglycemic clamps. Plasma glucose concentrations were clamped at ~8.6 mmol/l for 48 h in all subjects. In the saline control group, this was accomplished with a constant glucose infusion (55.5 μmol · kg^{-1} · min^{-1}). In the lipid/heparin group, glucose was infused at variable rates together with a constant infusion (4.3 μmol · kg^{-1} · min^{-1}) of fat (Liposyn II, Abbott, North Chicago, IL) and heparin (0.4 U · kg^{-1} · min^{-1}). Liposyn II is a 20% triglyceride emulsion containing 10% safflower and 10% soybean oil. In the variable glucose infusion group, blood samples were collected every 30 min, if necessary, to adjust the glucose infusion rate. In all subjects, blood samples were collected every 2 h for measurement of glucose, insulin, and C-peptide concentrations. The patients were fasting throughout the study but were allowed to drink water ad libitum. Plasma electrolytes were monitored every 24 h, body weight every 12 h, and fluid balance every 6 h. Fluid balance was maintained with infusion of normal saline. Potassium and magnesium were added to the glucose infusion as needed to maintain normal plasma concentration.

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BMI, body mass index; FFA, free fatty acid; GIR, glucose infusion rate; HGO, hepatic glucose output; ISR, insulin secretion rate; NIDDM, non-insulin-dependent diabetes mellitus; RIA, radioimmunoassay.

TABLE 1
Study subjects

Infusions	Fat/heparin	Saline
Sex (M/F)	6/0	5/1
Age (years)	29.0 ± 4.1	31 ± 3.2
Height (cm)	179 ± 2.9	178 ± 5.0
Weight (kg)	75.0 ± 2.0	82 ± 6.8
BMI (kg/m^2)	23.4 ± 0.4	25.9 ± 1.4

C-peptide kinetic studies. Either before or within 1 week of the studies, a 50 nmol i.v. bolus of biosynthetic human C-peptide (Lilly, Indianapolis, IN) was administered to each subject after an overnight fast and plasma C-peptide concentrations were measured at frequent intervals for 3 h as described by Polonsky et al. (13) and Van Cauter et al. (14).

Insulin secretory rates. The C-peptide kinetic parameters were used to calculate the insulin secretory rates for each time interval between successive blood samples during the hyperglycemic period by deconvolution of peripheral C-peptide concentration according to Eaton et al. (15) and Polonsky et al. (13,14). Plasma volume was assumed to be 4.1% of ideal body weight plus 1% of excess body weight (16).

Insulin clearance rates. Insulin clearance rates were determined for 24-h periods by dividing the areas under the curves of the insulin secretory rates by the areas under the curves of the serum insulin concentration.

Glucose utilization. Glucose utilization was estimated in all 12 subjects by using the glucose infusion rates (GIRs) needed to maintain the hyperglycemic clamps. In the lipid infusion group, we determined glucose turnover rates before (−1 to 0 h), after 1 day (23–24 h), and after 2 days (45–46 h) of fat infusion.

Glucose turnover. Glucose turnover was determined with 3-[^{3}H]glucose. The tracer infusion (40 μCi over 1 min followed by 0.4 μCi/min) was started 90 min before the measurements to ensure isotope equilibration. Glucose was isolated from blood for determination of 3-[^{3}H]glucose specific activity as described previously (17). Changes in specific activity during hyperinsulinemia were avoided by adding 3-[^{3}H]glucose to the unlabeled glucose that was infused at variable rates to maintain hyperglycemia (18). Rates of total body glucose appearance and disappearance were calculated using Steele's equation for steady-state conditions (19).

Hepatic glucose output (HGO). HGO was calculated as the difference between the isotopically determined rates of glucose appearance and the rates of glucose infused to maintain hyperglycemia during the clamps (GIRs).

Analytical procedures. Plasma glucose was measured with a glucose analyzer with the glucose oxidase method, and serum insulin was determined by radioimmunoassay (RIA) with a specific antibody that cross-reacts only minimally (<0.2%) with proinsulin (Linco, St. Charles, MO). C-peptide was determined by RIA. Electrolytes were measured at the Temple University Hospital Chemistry Laboratory. Plasma β-hydroxybutyrate was measured enzymatically (20).

Statistical analysis. All data are expressed as means ± SE. Statistical significance was assessed using analysis of variance and the two-tailed Student's *t* test when indicated.

RESULTS

Plasma glucose and FFAs. Mean clamp plasma glucose concentrations were 8.8 ± 0.04 mmol/l in the fat/heparin and 8.6 ± 0.6 mmol/l in control studies (NS) (Fig. 1). Mean clamp plasma FFA concentrations were 1,237 ± 113 μmol/l in the fat/heparin and 132 ± 23 μmol/l in the control group ($P <$ 0.001).

Insulin secretion rates. Basal insulin secretion rates (ISRs) were 74 ± 9 and 57 ± 14 pmol/min mmol/l in the lipid/heparin and control groups, respectively (NS) (Fig. 2). In response to hyperglycemia, mean 48-h ISRs rose significantly higher in the fat/heparin group (to a mean 48-h concentration of 352 ± 38 pmol/min) than in the control group (mean 48-h concentration 241 ± 43 pmol/min, $P <$ 0.05) and remained higher throughout the study.

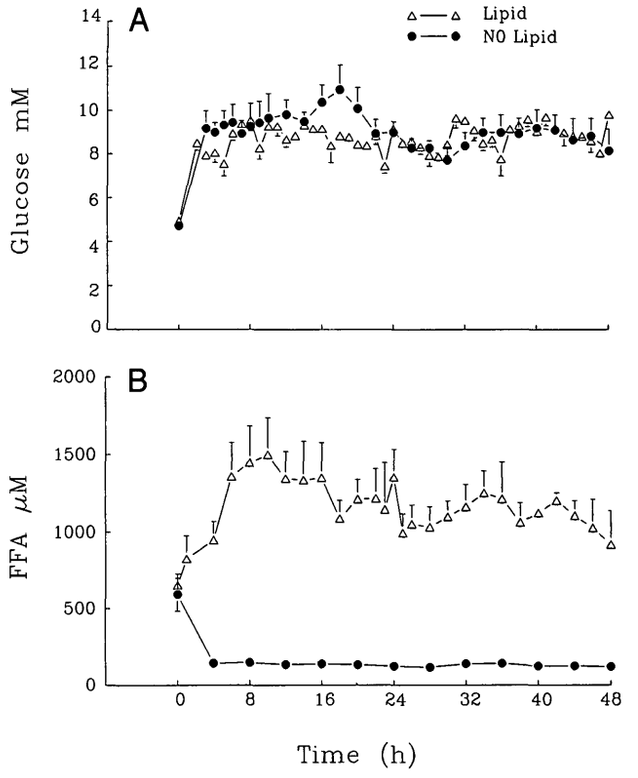

FIG. 1. ***A:*** **Plasma glucose concentrations during 48 h of hyperglycemic clamping. Six subjects received Liposyn II (4.3 μmol/min) plus heparin (0.4 U/min) (△). Six control subjects received saline (●).** ***B:*** **Plasma FFA concentrations in the same subjects. Data are means ± SE.**

Insulin clearance rates. Mean 24-h insulin clearance rates during day 1 were 1.02 ± 0.4 l/min during fat/heparin and 0.97 ± 0.4 l/min during saline infusion (NS) (Fig. 3). During day 2, insulin clearance rates declined nonsignificantly to 0.73 ± 0.27 and 0.72 ± 0.16 l/min, respectively, in the two groups.

Serum C-peptide and insulin. Mean 24-h clamp plasma C-peptide concentrations during days 1 and 2 were 2.08 ± 0.26 and 2.20 ± 0.28 nmol/l, respectively, in the fat/heparin group and 1.55 ± 0.22 and 1.49 ± 0.20 nmol/l, respectively, in the control group. C-peptide concentrations were significantly higher in the fat/heparin group when compared with the control group during both days ($P < 0.01$).

Mean 24-h serum insulin concentrations during day 1 were 331 ± 99 pmol/l during fat/heparin and 258 ± 61 pmol/l during saline infusion. During day 2, insulin concentrations

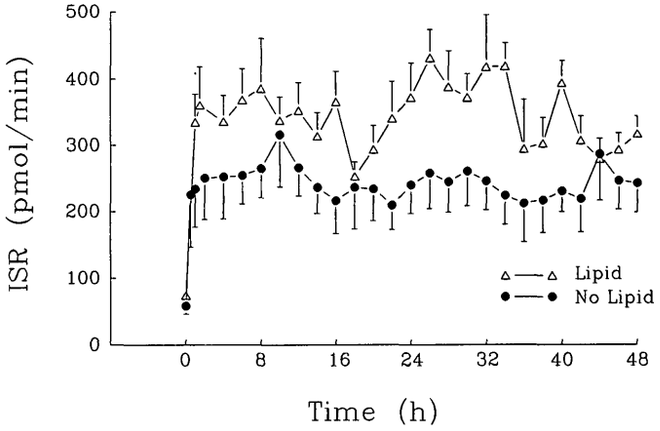

FIG. 2. ISRs during hyperglycemic clamping with and without fat/heparin infusion. Symbols and number of subjects as in the legend to Fig. 1.

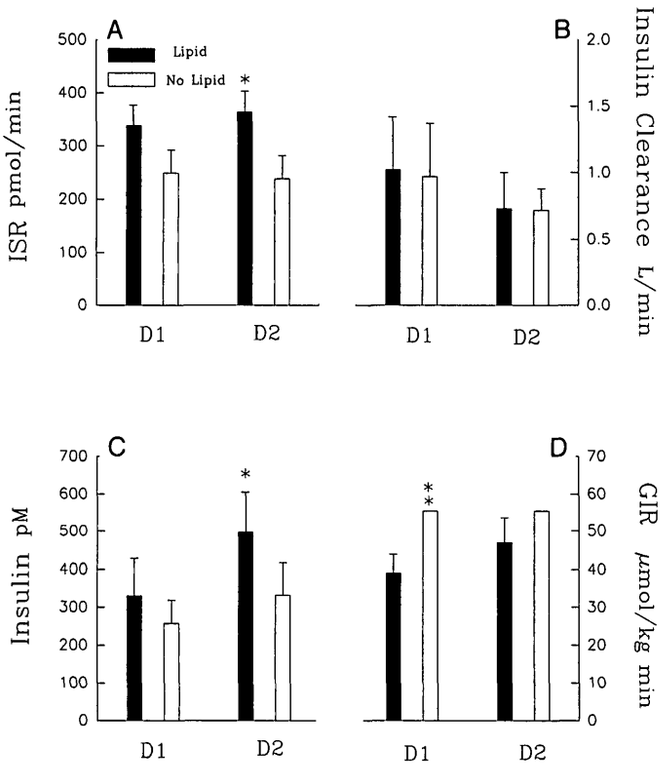

FIG. 3. Mean ISRs (*A*), insulin clearance rates (*B*), serum insulin concentrations (*C*), and GIRs (*D*) during day 1 (D1) and day 2 (D2) of 48-h hyperglycemic clamping without and with fat/heparin infusion. Number of subjects as in the legend to Fig. 1. *$P < 0.05$; **$P < 0.02$ comparing fat/heparin with control subjects.

were 497 ± 108 and 332 ± 86 pmol/l, respectively, in the two groups. The differences between the fat/heparin group and control subjects were significant only during day 2 ($P < 0.05$) (Fig. 3).

GIRs. Mean GIR was significantly lower in the fat/heparin group than in the control group during day 1 (39.1 ± 4.9 vs. 55.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$) (Fig. 3). During day 2, the difference between the two groups became smaller and statistically nonsignificant (47.1 ± 6.5 vs. 55.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$).

HGO. HGO in the fat/heparin group was 6.2 ± 2.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ after 24 h and 6.7 ± 2.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ after 48 h of lipid/heparin infusion.

DISCUSSION

Prolonged elevation of FFAs and insulin secretion. The main objective of this study was to determine whether prolonged elevation of plasma FFA concentration inhibited glucose-stimulated insulin secretion in normal subjects, i.e., whether the inhibitory results of FFA on insulin secretion obtained in vitro by others (7,8) could be reproduced in vivo. The results showed that this was not the case. Instead, we found that high plasma FFA concentrations were associated with mean 48-h insulin secretion rates that were 46% higher than insulin secretion rates associated with low FFA concentrations. Moreover, insulin secretion rates remained persistently elevated during the 48 h of fat infusion.

The possibility that the stimulation of insulin secretion in our studies was caused by substances other than fatty acids was unlikely. Plasma triglycerides and glycerol concentrations rose during the fat infusions, but neither one is an insulin secretagogue (21). β-hydroxybutyrate, a weak insulin secretagogue (22), actually decreased during fat infusion (from 0.071 ± 0.025 to 0.031 ± 0.01 mmol/l), whereas glucose concentrations were similar in both groups. In addition, release of insulin stimulatory gut factors, including gastrointestinal polypeptide, cholecystokinin, and glucagon-like peptide I, were avoided by intravenous infusion of fat. On the other hand, acute stimulation of insulin secretion by FFAs has been well established in humans and in experimental animals in vitro as well as in vivo (1–6). Moreover, Crespin et al. (4) demonstrated direct acute pancreatic effects of fatty acids by infusing FFAs into the pancreatic artery of dogs. Thus, our data are most compatible with the notion that fatty acids stimulated insulin release by direct action on the pancreas.

The differences between our results and those of Sako and Grill (7) and Zhou and Grill (8) were more apparent than real. These investigators, in fact, showed that in hyperglycemic (~22 mmol/l) rats, plasma insulin levels were ~40% higher after 48 h of intravenous fat infusion than after 48 h of saline infusion (7). Thus, their in vivo data as well as ours showed that fat infusion potentiated glucose-stimulated insulin secretion. The inhibitory effect of fat was only seen in vitro when the pancreas was removed from these rats and perfused with 27.7 mmol/l glucose (7). Why glucose-stimulated insulin secretion was potentiated by FFAs in vivo but was inhibited in vitro remains unknown. Nevertheless, the finding that in vivo physiological elevations of plasma fatty acid levels stimulated insulin secretion unabated for 48 h casts doubt on the physiological or pathophysiological relevance of the inhibitory effects seen in vitro.

Prolonged elevation of FFAs and peripheral glucose uptake. Our study also provided an opportunity to assess the effects of prolonged elevation of fatty acids on peripheral glucose utilization. During day 1, GIR (an index of peripheral glucose uptake) was lower during high FFA concentration (fat infusion) than during low FFA concentration (control). The difference became smaller and statistically nonsignificant during day 2. Because fat infusion has been shown to increase insulin-inhibited HGO (11), the inhibitory effect of fat on true glucose uptake (which consists of GIR plus HGO) was probably slightly smaller. HGO, unfortunately, was measured only in the lipid infusion group, where it was ~7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ on both days 1 and 2. Assuming that HGO in the saline control subjects was suppressed completely (a worst-case scenario), glucose uptake remained significantly inhibited during the first 24 h of fat infusion (45.3 ± 4.3 vs. 55.5 ± 0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.02$). On day 2, however, glucose uptake in fat-infused subjects increased and the difference in glucose uptake between lipid infusion and control subjects disappeared (53.8 ± 6.9 vs. 55.5 ± 0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, NS). These results confirmed previous reports from our laboratory and others showing that fat infusion caused acute insulin resistance (9–11). The results extend these findings by demonstrating that under hyperglycemic conditions, these inhibitory actions of fat lasted only ~24 h in these healthy subjects. The most likely reason for the improved glucose uptake after 24 h of fat infusion was an increase in circulating insulin, which rose from 331 ± 99 pmol/l on day 1 to 497 ± 108 pmol/l on day 2 ($P < 0.05$). Because insulin secretion did not change (339 vs. 364 pmol/min), serum insulin may have increased because insulin clearance decreased (1.02 ± 0.4 on day 1 to 0.73 ± 0.23 l/min

on day 2), although the decrease failed to reach statistical significance. In addition, prolonged hyperinsulinemia may have contributed to the enhanced glucose uptake on day 2 in accordance with observations by others (23,24).

In summary, in healthy subjects, prolonged (48 h) elevation of plasma FFAs (~1 mmol/l) persistently potentiated glucose-stimulated insulin secretion. In addition, this elevation inhibited peripheral glucose uptake, i.e., produced insulin resistance. The insulin resistance, however, was transient and disappeared after ~24 h of continued insulin hypersecretion. One would suspect, therefore, that elevation of FFAs causes a longer-lasting increase in insulin resistance in subjects who are unable to produce a compensatory increase in insulin secretion.

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